



# Gene Therapy in the Clinic - the CF Odyssey

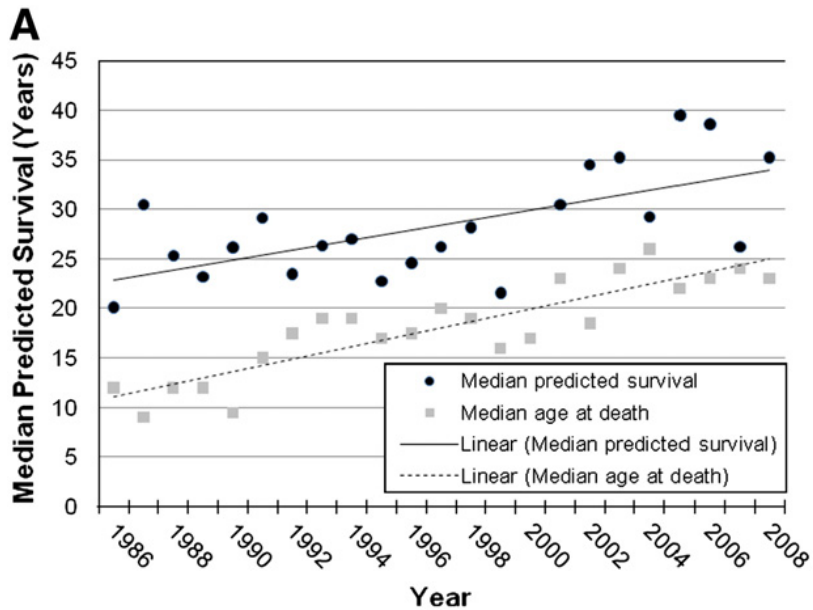
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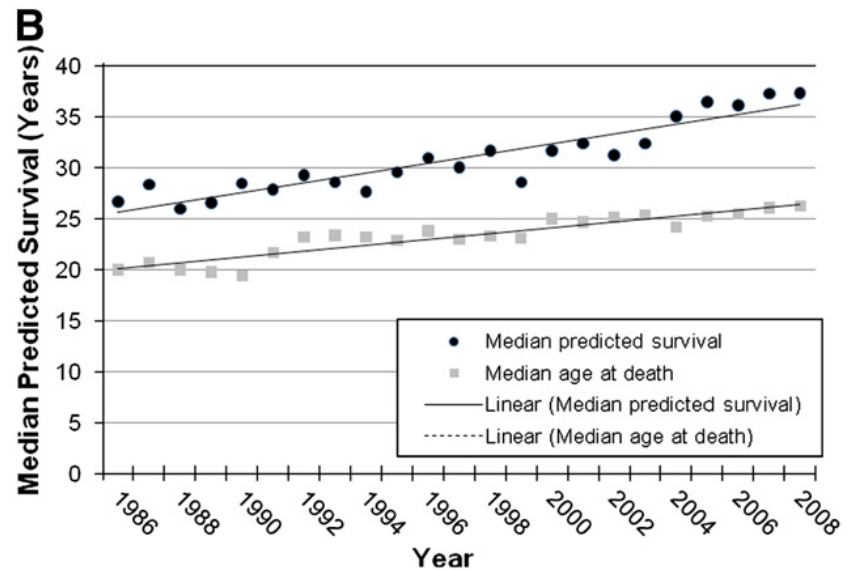
# CF: Epidemiology

- Incidence: 1:1600 (IRL)
- CF gene mutation frequency 1:17 (IRL)
- CF census 2005 – 1182 patients (IRL)
- 55% Children/ 45% Adults (IRL)
- Mortality ~ 16/ per annum (IRL)
- Current Survival 2007 – 38 years (US)

# Median Survival



Ireland



USA

# Multiple Manifestations of CF

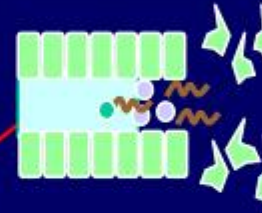
## Sinopulmonary

Air trapping, bronchiectasis, mucus plugging, chronic bacterial/fungal infections, atelectasis, bronchial cysts, pneumothorax, opacification of sinuses<sup>1-3</sup>



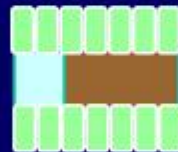
## Pancreatic & Hepatic

Exocrine PI, pancreatitis, cirrhosis, portal hypertension, cholelithiasis, steatosis<sup>1,4</sup>



## Gastrointestinal

Meconium ileus, distal intestinal obstruction syndrome<sup>1</sup>



## Endocrinologic

Insulin resistance, diabetes<sup>5</sup>

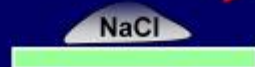
## Reproductive

CBAVD, reduced fertility<sup>1</sup>

## Others

Digital clubbing,<sup>6</sup> metabolic alkalosis<sup>1</sup>

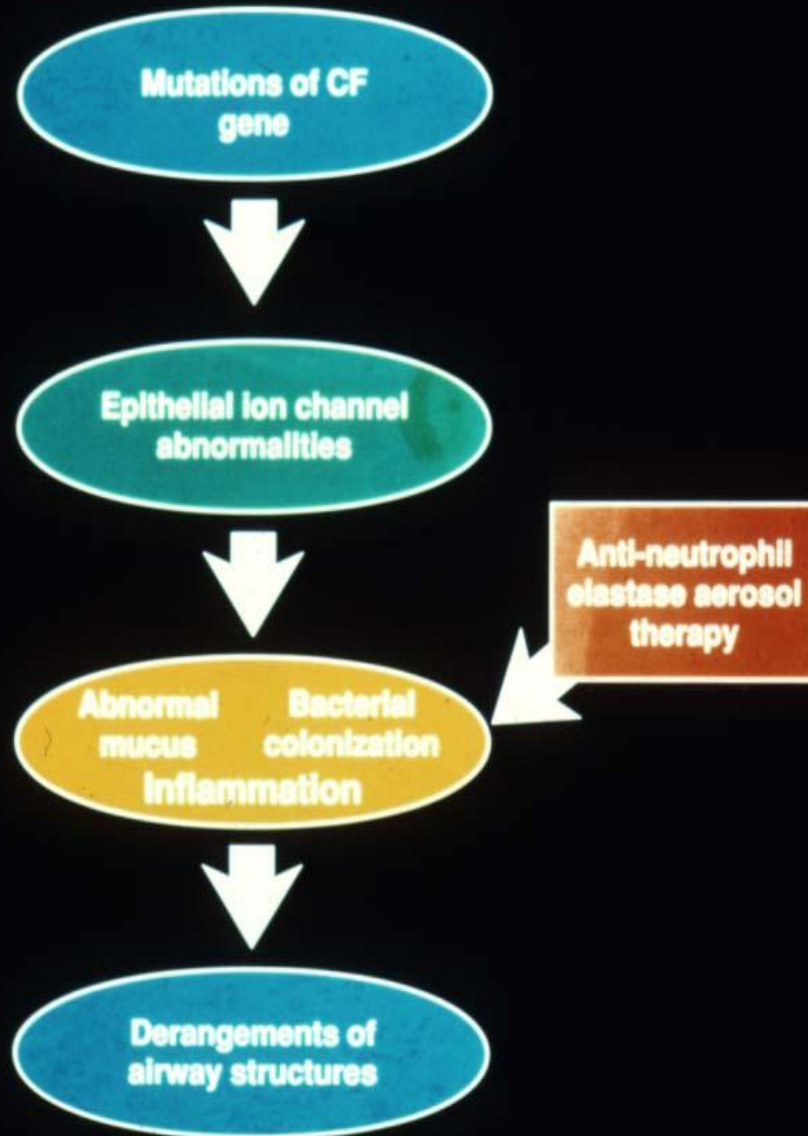
## Salt Balance<sup>1</sup>



CBAVD=congenital bilateral absence of the vas deferens; PI= pancreatic insufficiency.

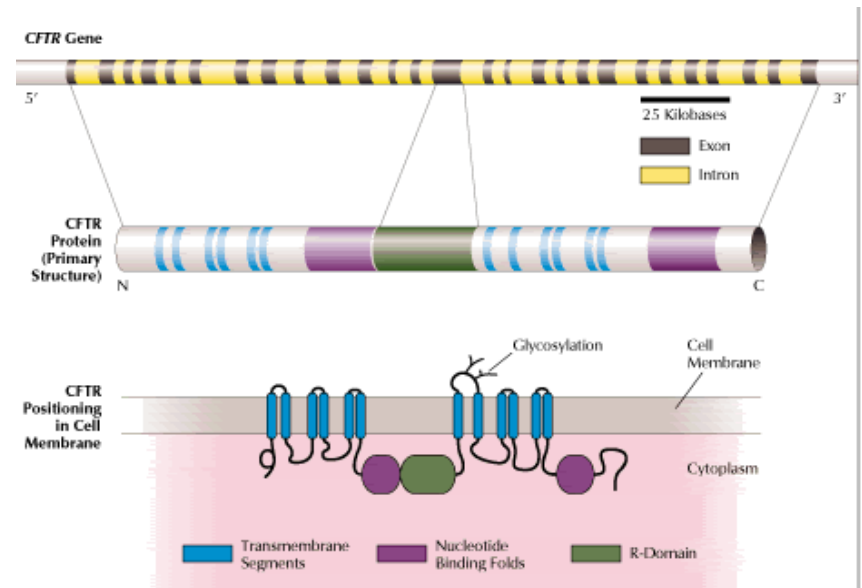
1. Davis PB. *Am J Respir Crit Care Med*. 2006;173(5):475-482. 2. Ramsey BW. *Proc Am Thorac Soc*. 2007;4(4):359-363. 3. Tiddens HA, de Jong PA. *Proc Am Thorac Soc*. 2007;4(4):343-346. 4. Colombo C, et al. *Pediatr Gastroenterol Nutr*. 2006;43(suppl1):S49-S55. 5. Costa M, et al. *Diabetes Metab*. 2005;31(3 pt 1):221-232. 6. Augarten A, et al. *Pediatr Pulmonol*. 2002;34(5):378-380.

# THE THERAPY OF CYSTIC FIBROSIS



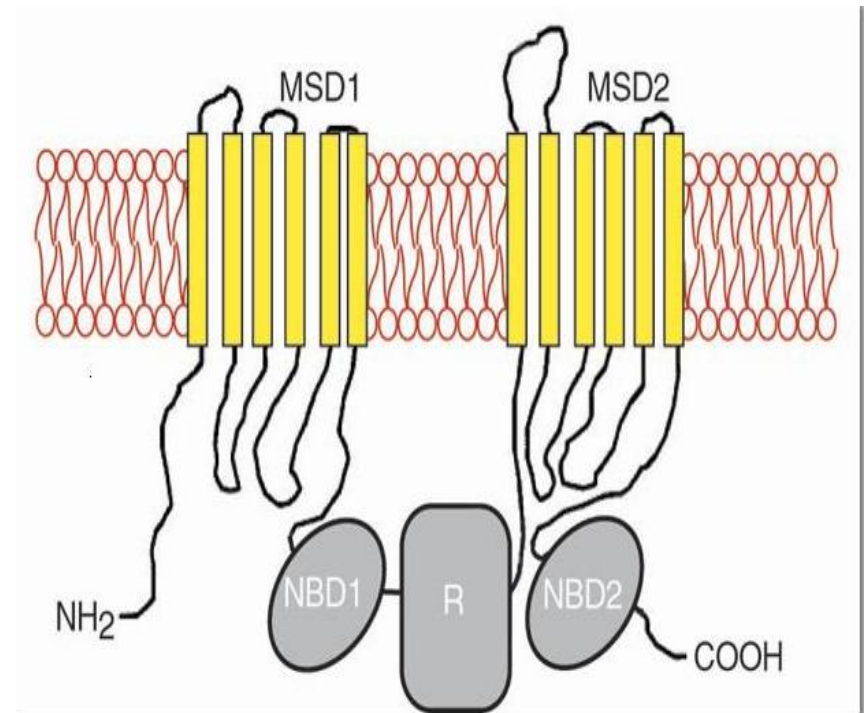
# CFTR gene

- Discovered 1989
- 27 exon, 250 kilobase segment of chromosome 7
- mRNA 6.5kb, including 4.5 kb coding exons and 2kb untranslated 3' tail
- Encodes a 1480 protein
- Membrane bound glycoprotein a member of ABC transporter family



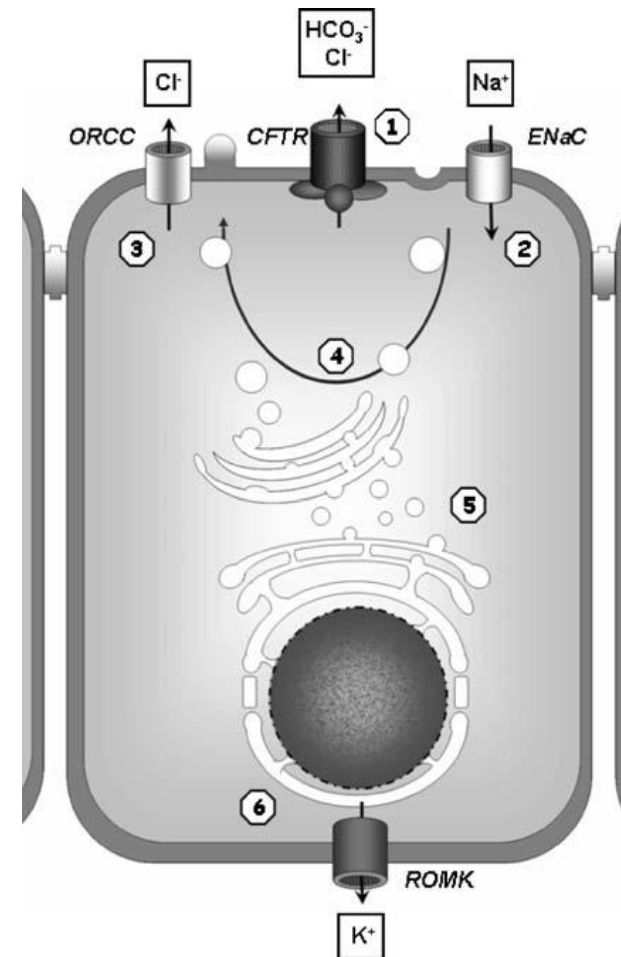
# CFTR protein

- Two membrane spanning domains
- Followed by nucleotide binding domain
- NBD1 and NBD2 bind ATP form heterodimer, channel opens Cl-flows
- R domain
  - Sites phosphorylated in cAMP dependent manner by PKA
  - Phosphorylation by PKA necessary for activation and gating following ATP binding



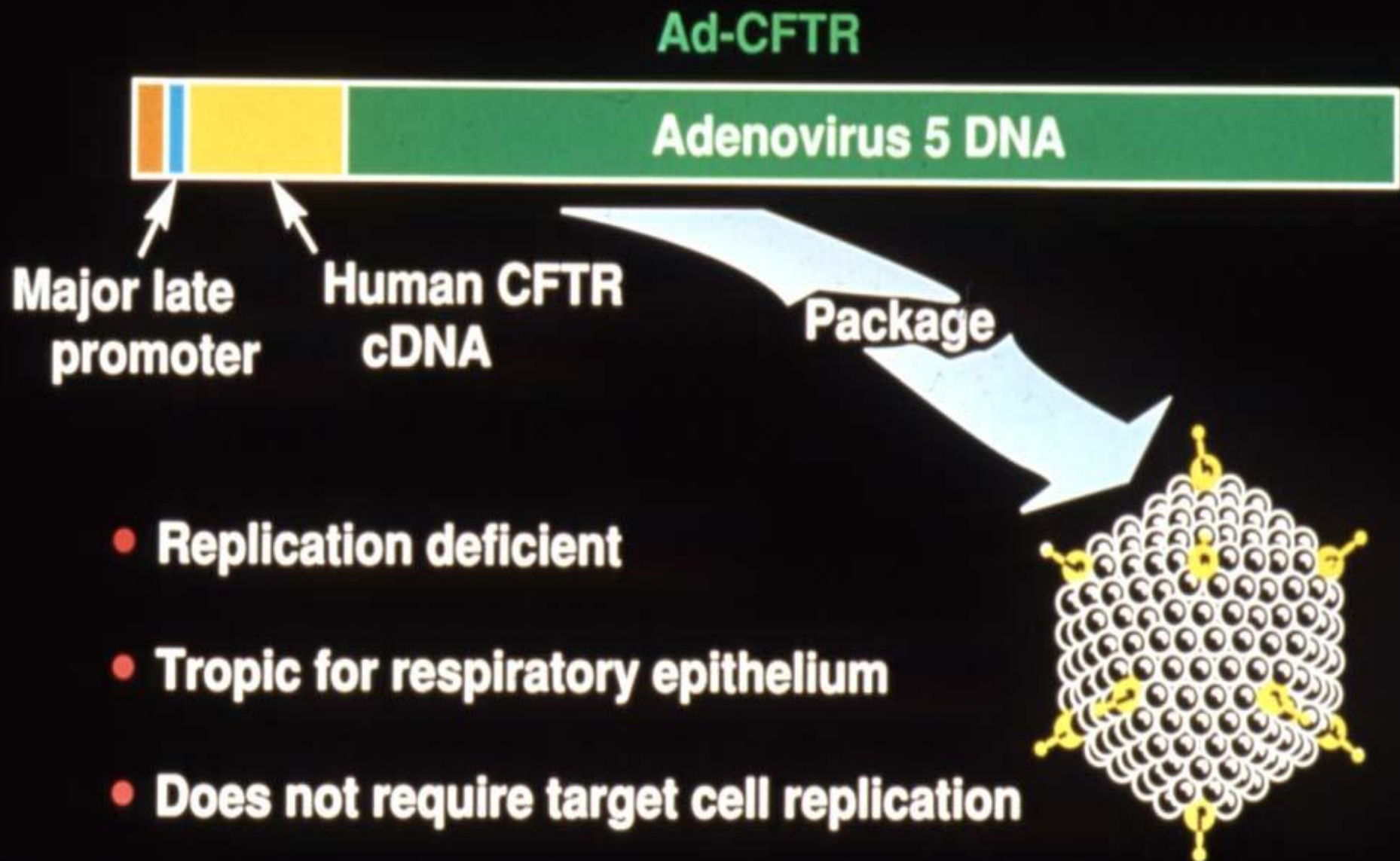
# CFTR function and interactions

- cAMP regulated Cl<sup>-</sup> channel facilitating release of Cl<sup>-</sup> , HCO<sub>3</sub><sup>-</sup> and ATP
- ATP downregulates ENaC activity
- CFTR positively regulates ORCC
- In absence of CFTR
  - Excess Na absorption plus water
  - Decreased Cl-fluid depletion airways



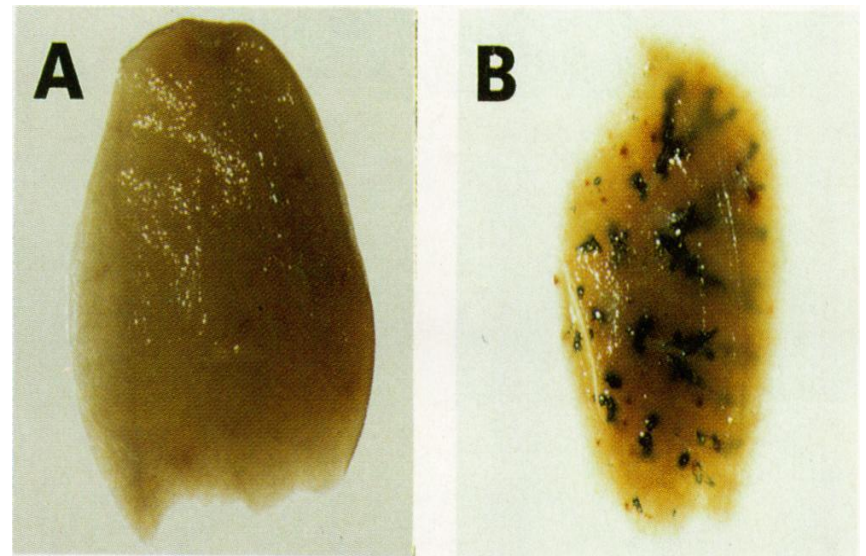


# RECOMBINANT ADENOVIRUS VECTOR



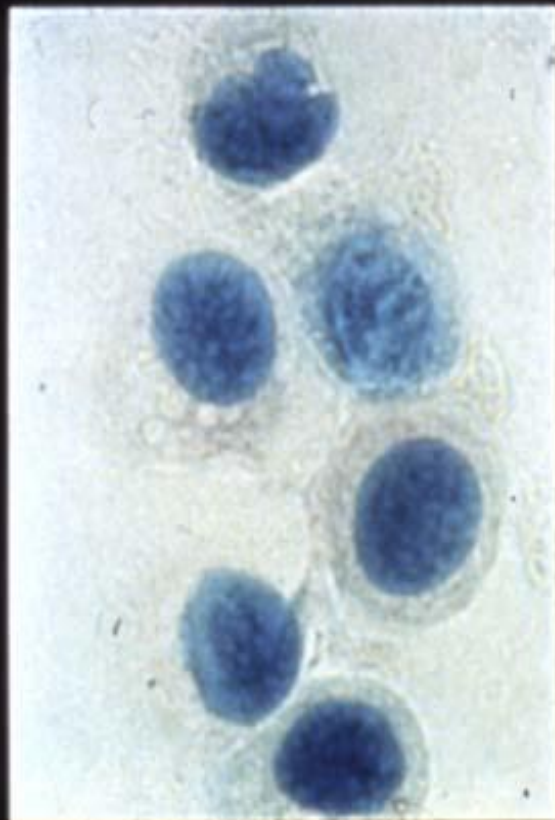
# Pre clinical safety studies

- Airway epithelium from humans
- Cotton rat
  - Intratracheal
  - Nasal
- Non human primates
- No major sustained inflammation
- Distribution normal and widespread

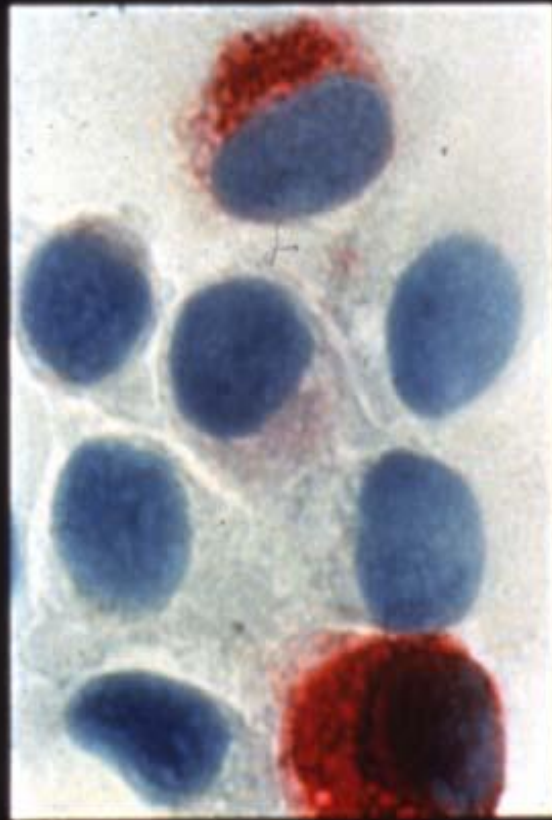


# Ad-CFTR MEDIATED EXPRESSION OF NORMAL CFTR PROTEIN IN CF AIRWAY CELLS

**Uninfected**



**+ Ad-CFTR**



# CF Gene Therapy Protocol

**Target** : Nasal and airway epithelia

**Vector** : AdCFTR

E1<sup>-</sup>E3<sup>-</sup> adenovirus

Major late promoter/ tripartite leader

Normal CFTR cDNA

**Protocol** : 1 dose to nose (1 side)

↓  
1 - 2 days

1 dose to airways (1 lobe)

**Doses** : 9 individuals

Nose  $2 \times 10^5 - 10^8$  pfu (0.2ml)

Airway  $2 \times 10^6 - 10^9$  pfu (5 or 20ml)

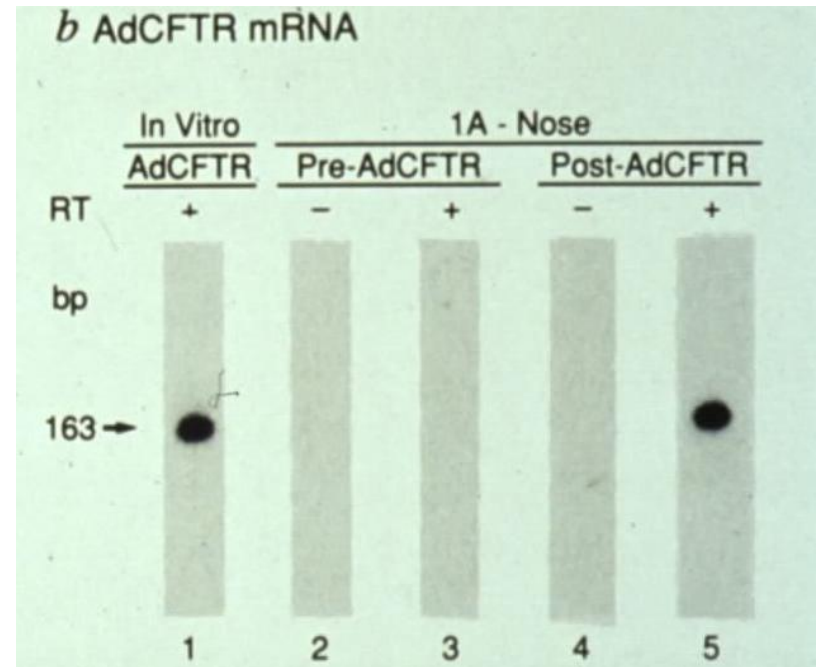
# Detection of AdCFTR DNA

- Specific amplification of AdCFTR DNA by PCR
- Southern blot hybridized with nested probe



# ADCFTR-derived CFTR mRNA

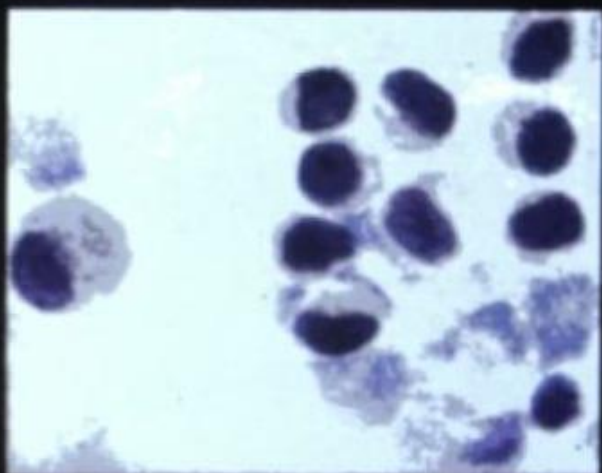
- Epithelial Cell RNA reverse transcribed
- PCR specific for ADCFTR-derived mRNA
- Southern analysis with  $^{32}\text{P}$  labelled nested probe



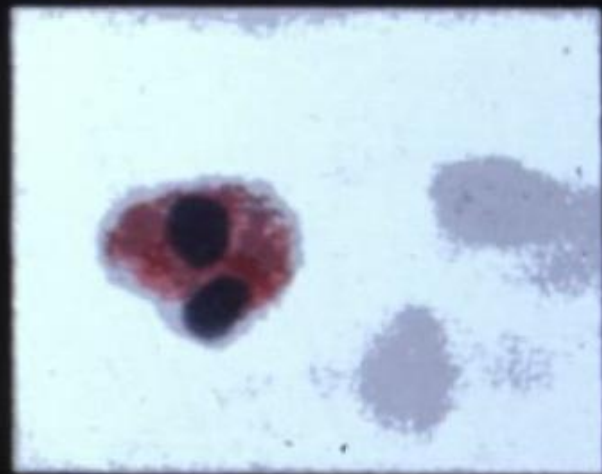
# Immunocytochemical Detection of Human CFTR Protein in Nasal Epithelial Cells

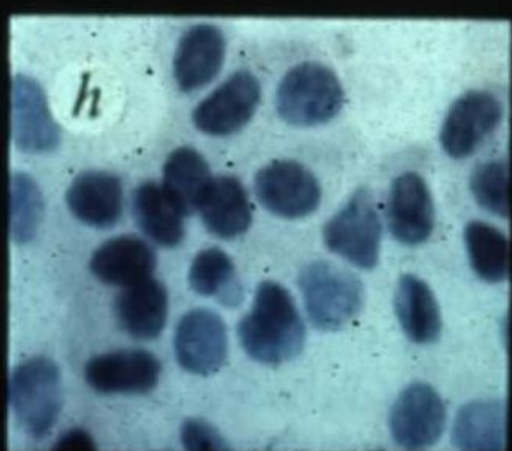
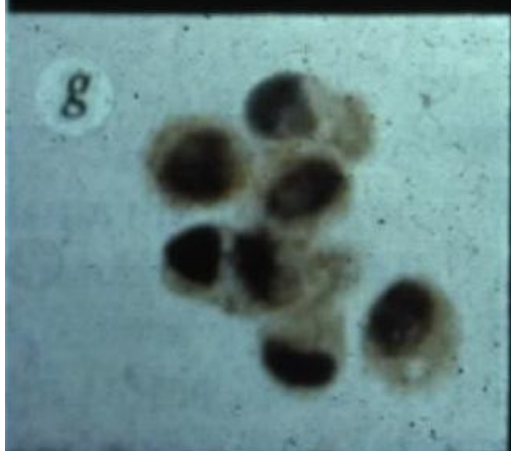
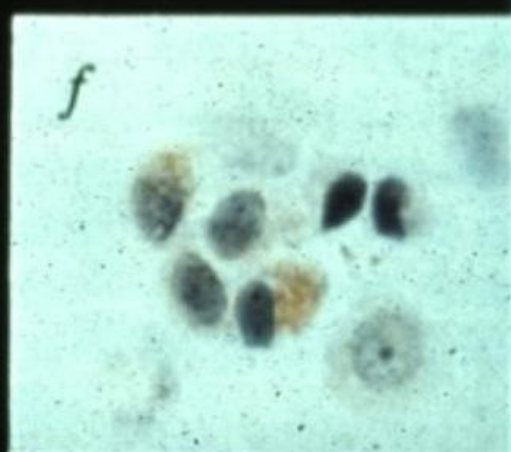
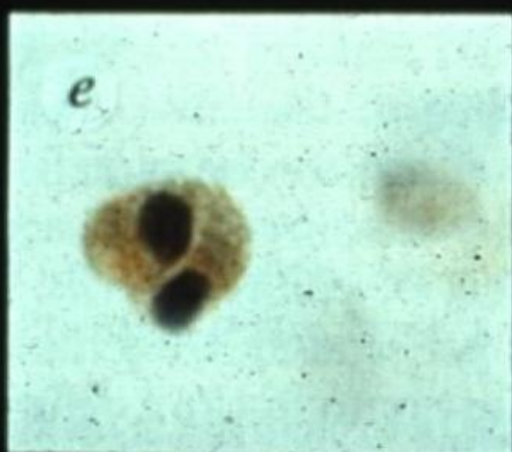
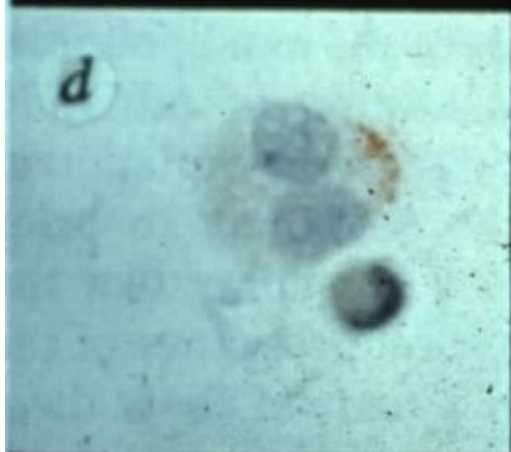
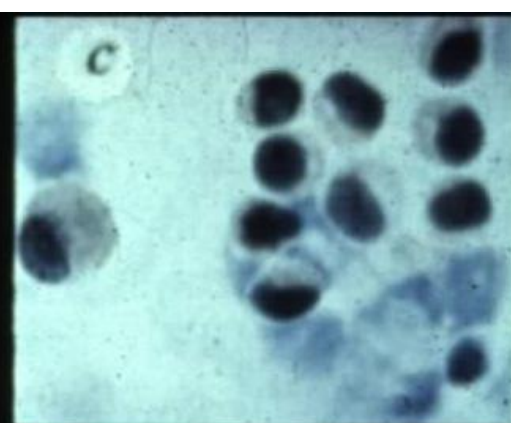
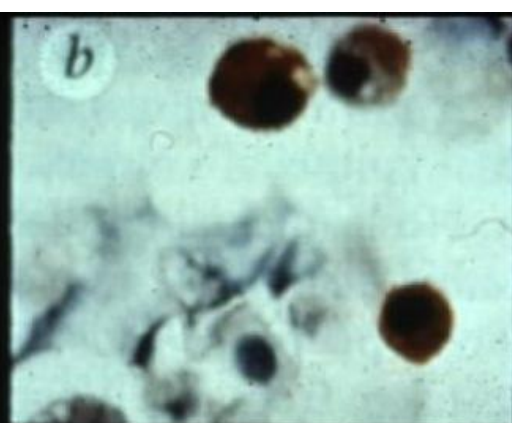
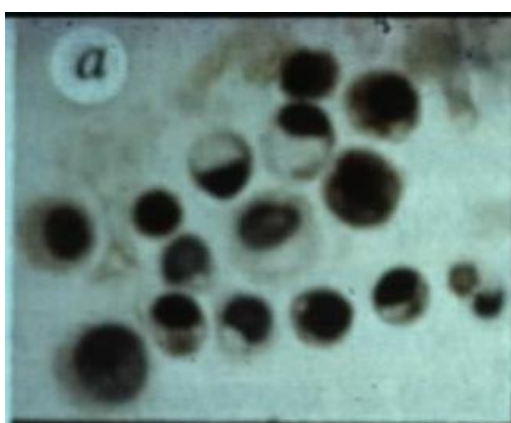
- Nasal epithelium obtained by brushing turbinate before and 2 d after administration of AdCFTR
- Anti-human CFTR monoclonal Ab

Before AdCFTR



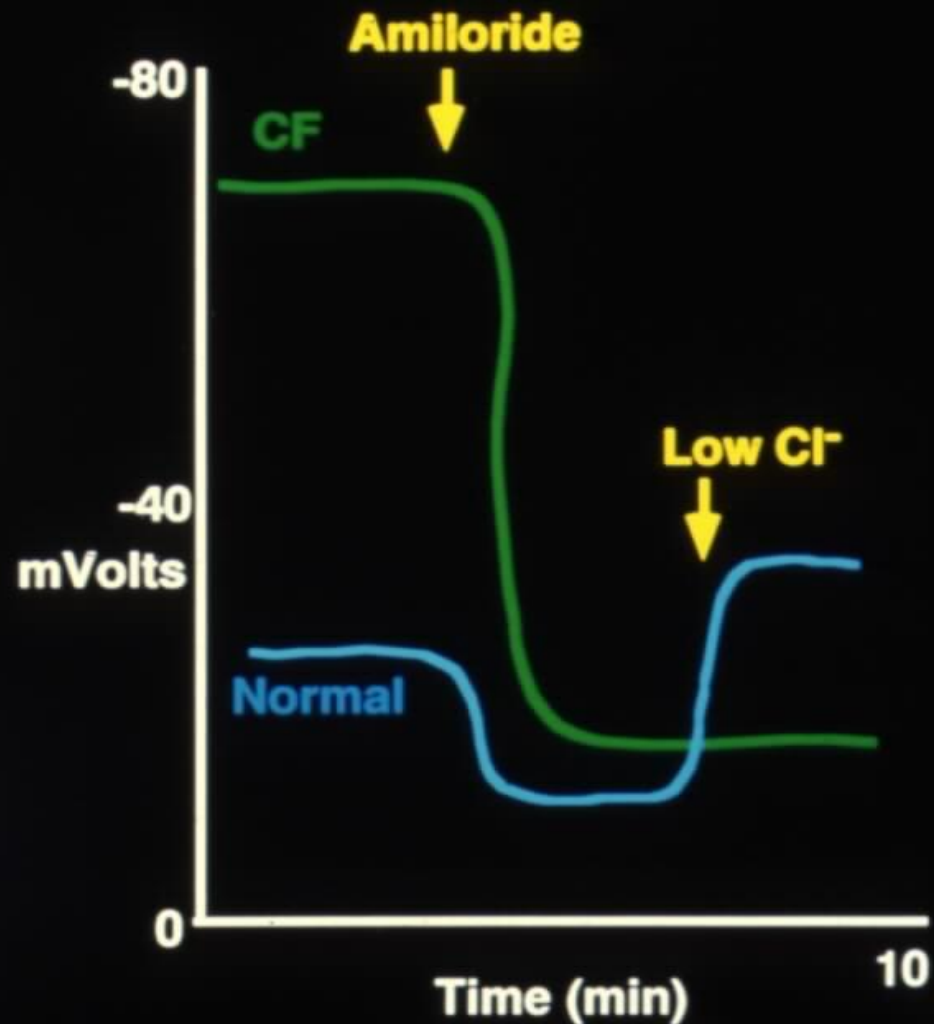
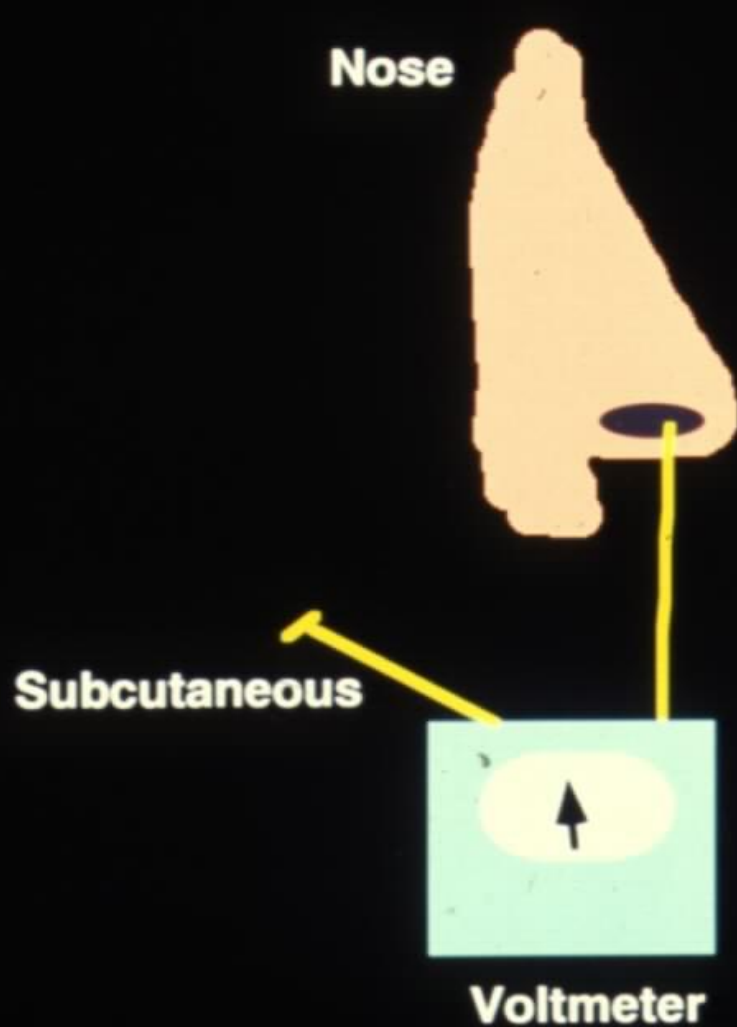
After AdCFTR



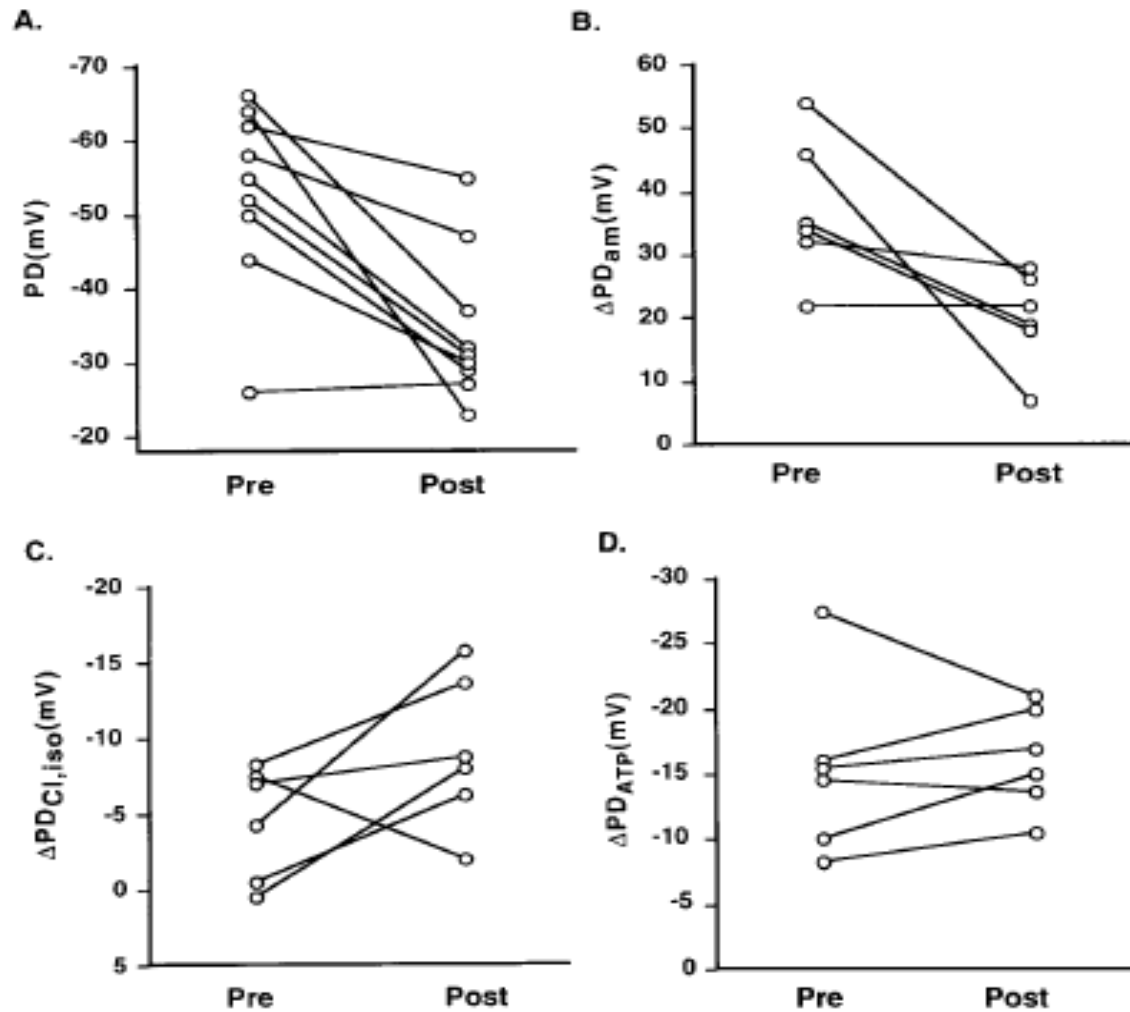


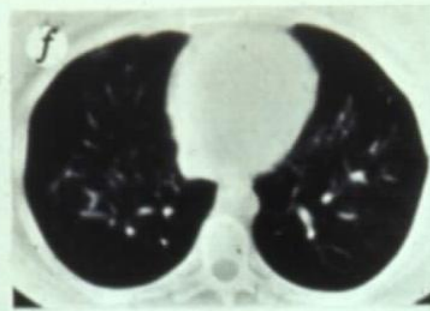
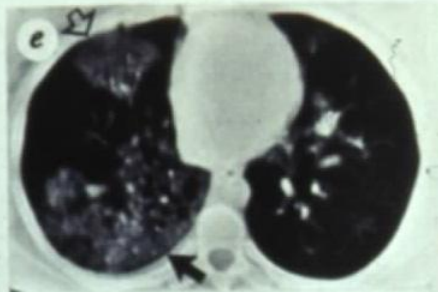
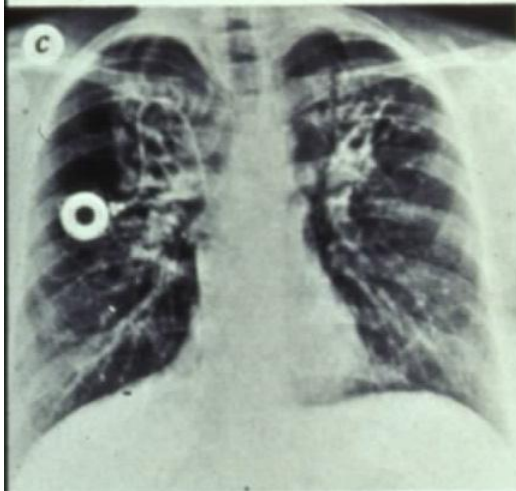
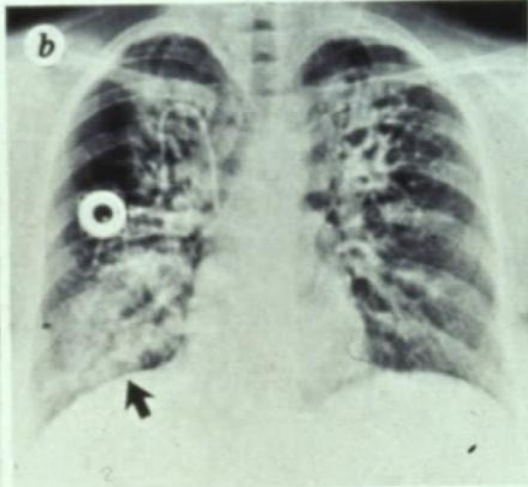
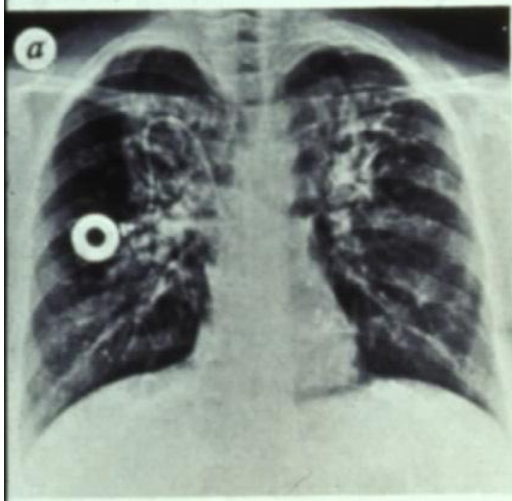


# CF Airways are Impermeable to $\text{Cl}^-$



# Nasal PD

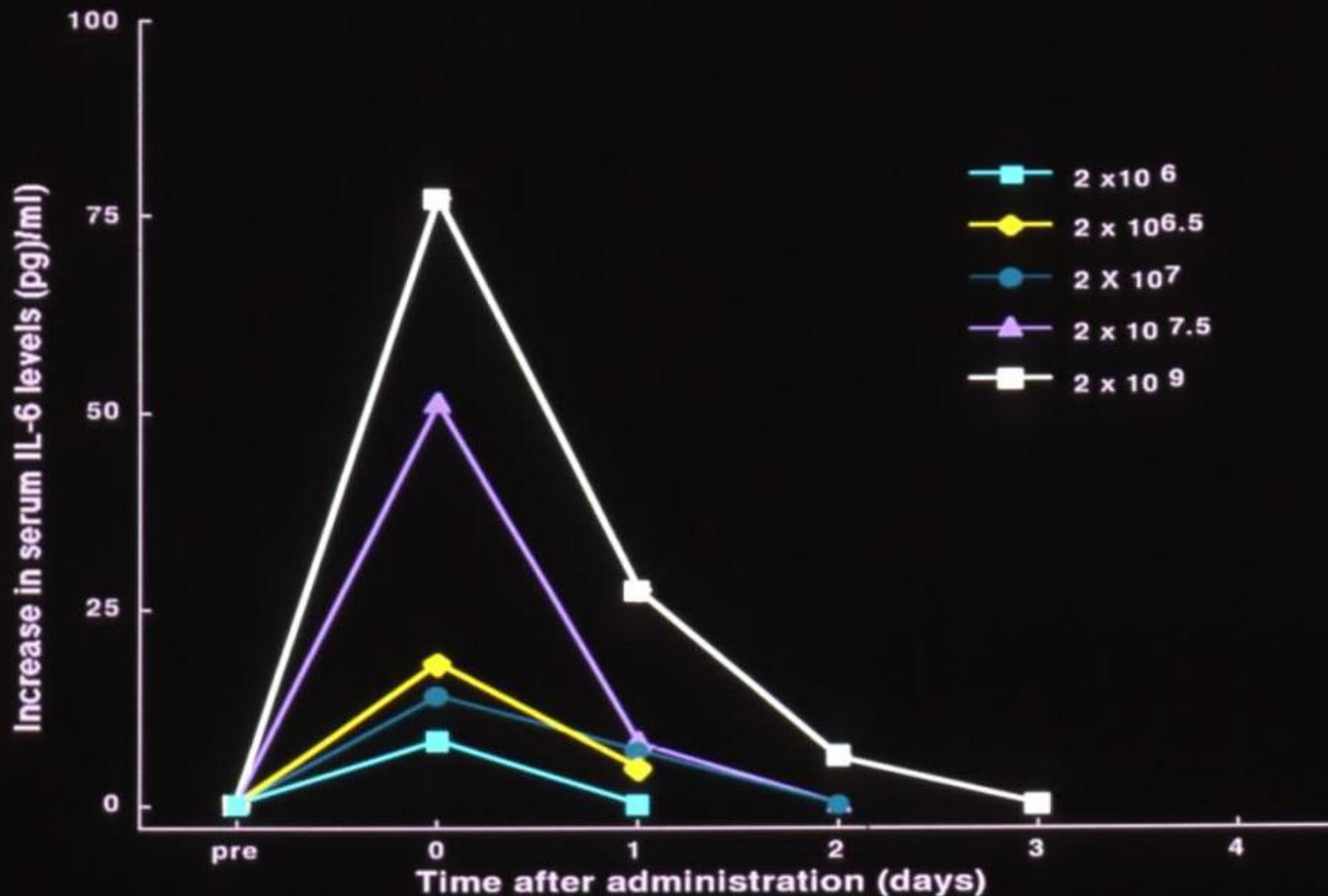




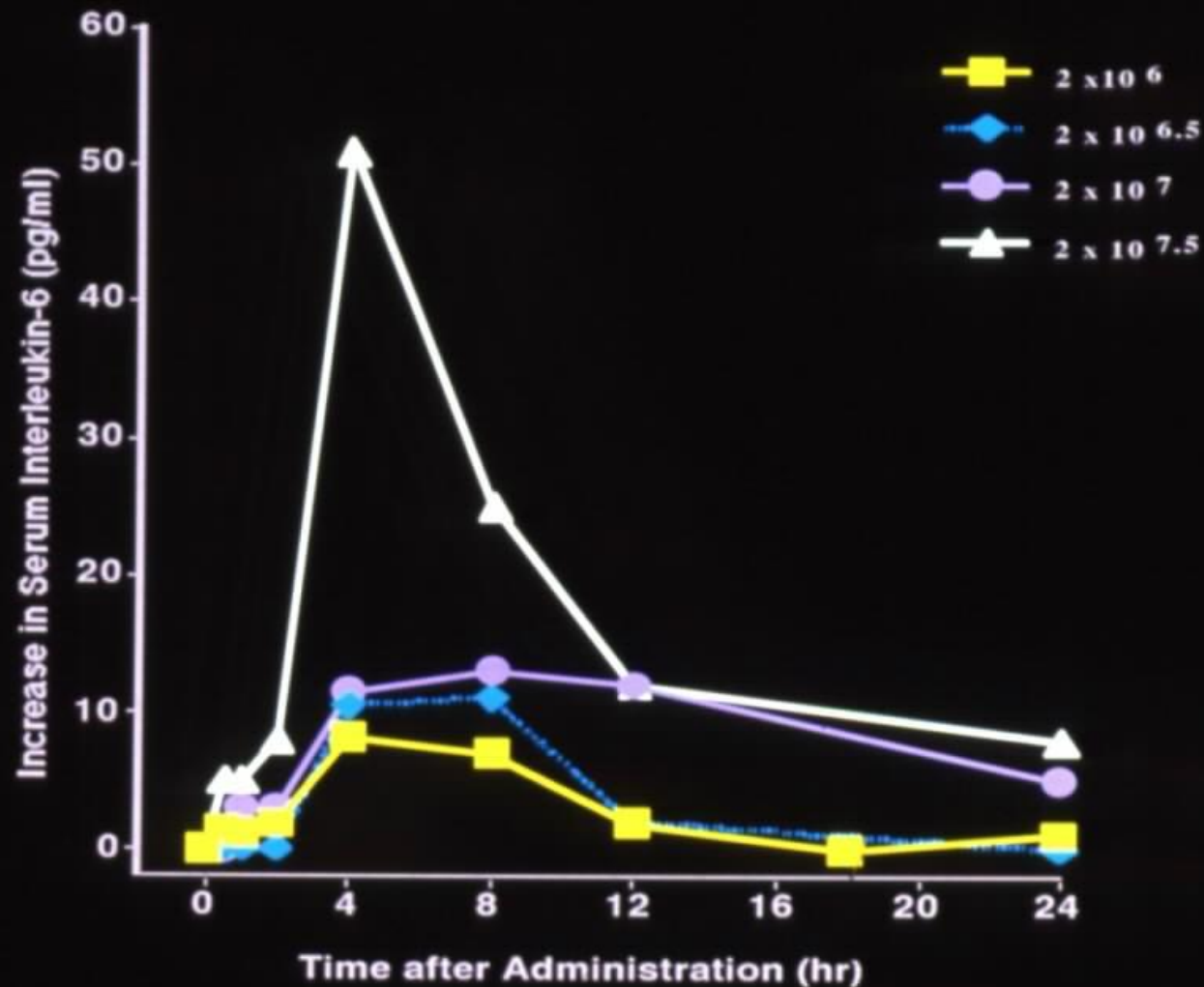
## **Cytokine - Related Inflammatory Syndrome Induced by Airway Administration of AdCFTR**

- **Systemic - 12 hrs to 6 days**
- **Local - alveolar, not airway**
- **Related to interleukin-6**
  - **Detected in serum by 4 hr**
  - **Persists over days**
  - **Dose-related**
  - **Consistent syndrome**

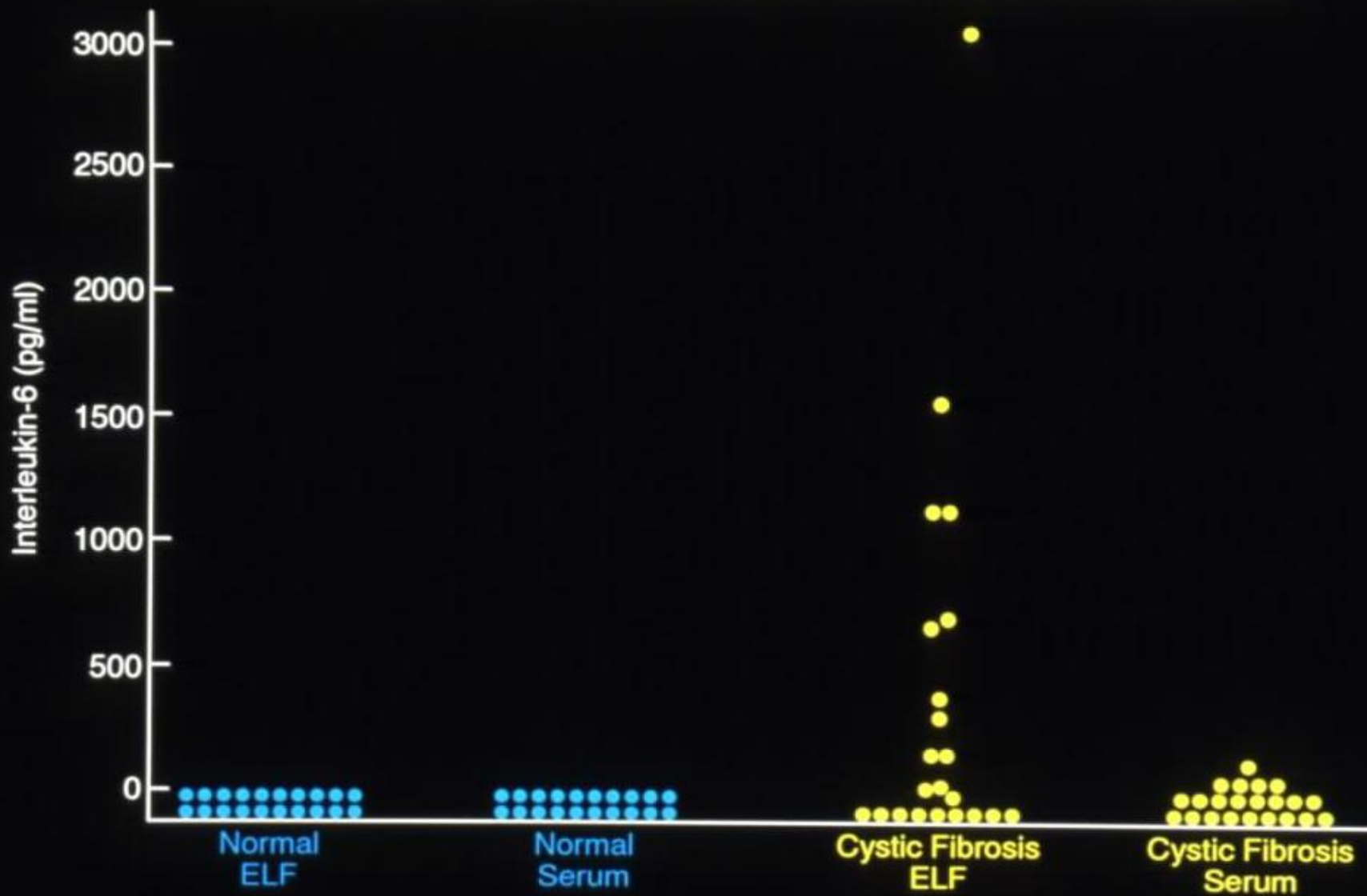
# Serum Levels of Interleukin-6 Following Administration of AdCFTR



# Dose-Related Serum Interleukin-6 Levels in First 24 Hours Following AdCFTR Administration



## Serum and Lung Epithelial Lining Fluid Levels of Interleukin-6 in Normals and Individuals with Cystic Fibrosis



# Adenovirus Vector-Induced Release of Interleukin-6 by Inflammatory Cells on the Respiratory Epithelial Surface

Inflammatory cells recovered by lavage



Cystic fibrosis  
n=3

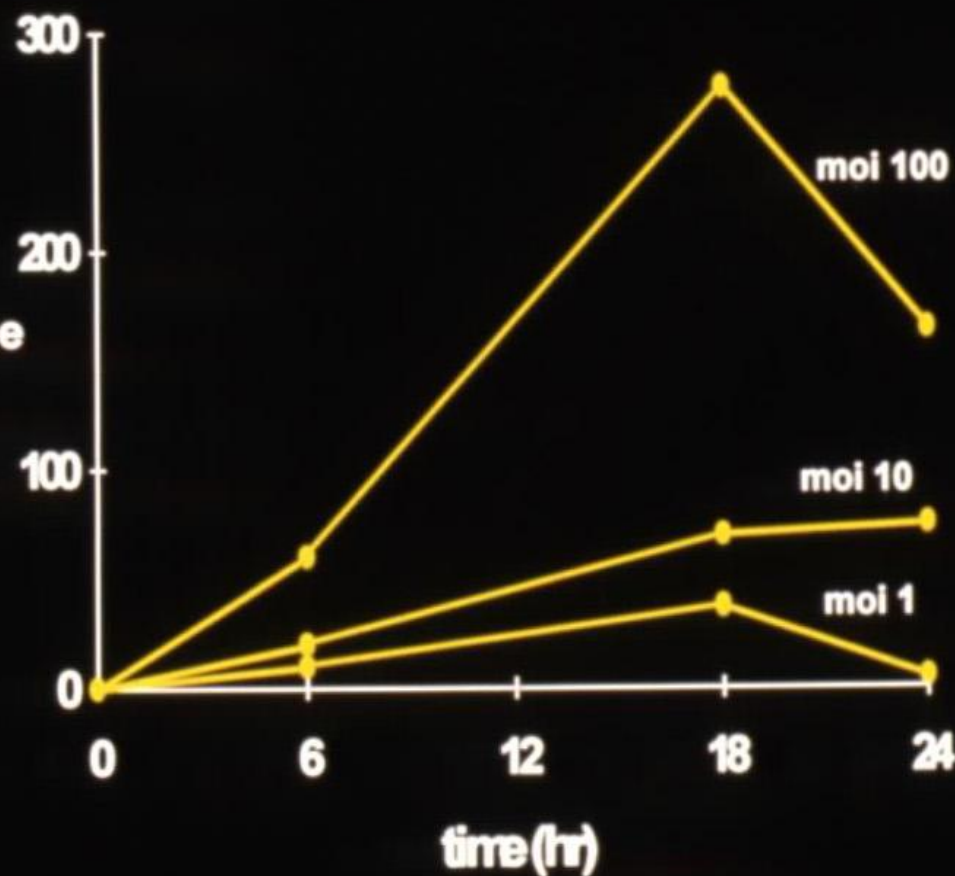
1-24 hr



**Evaluate**

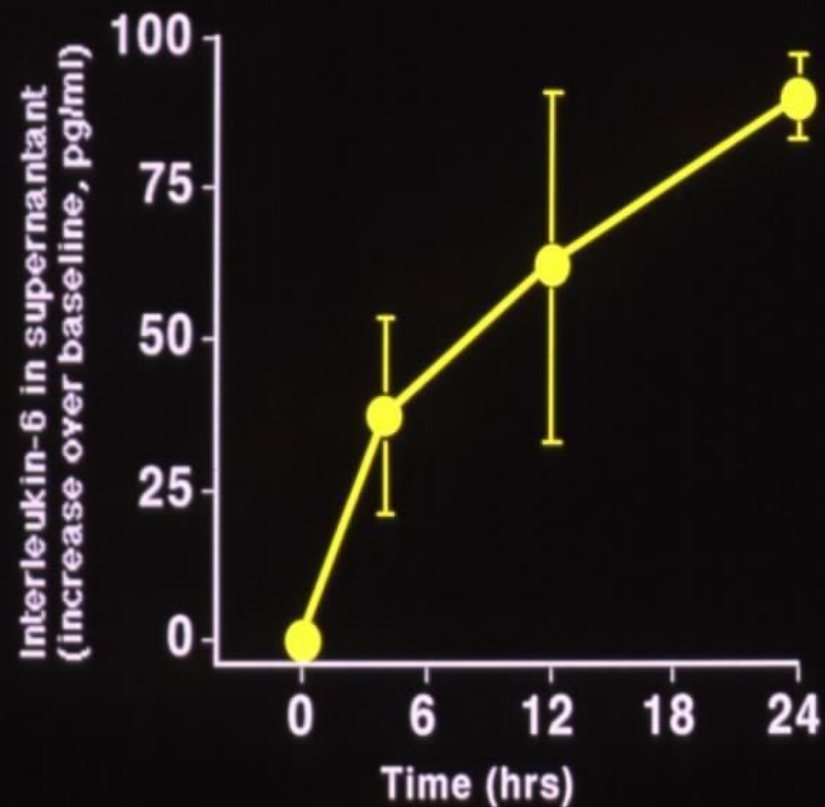
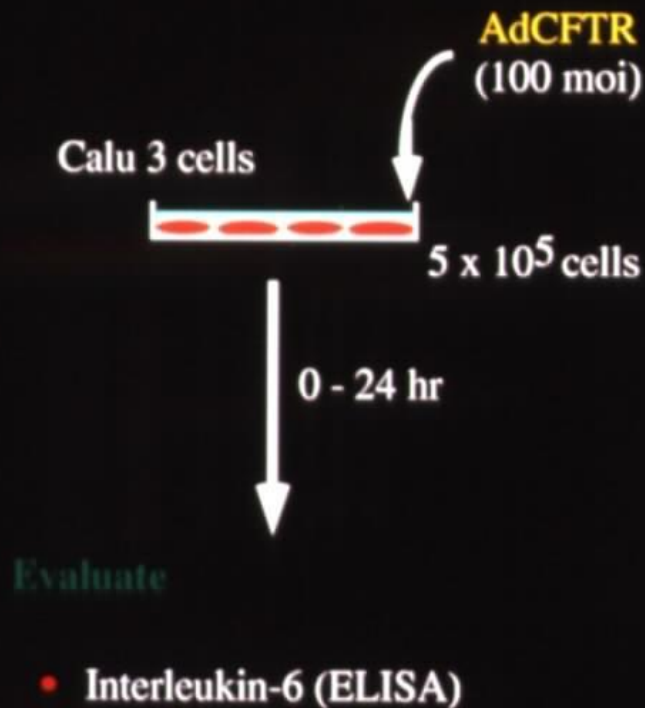
- Supernatant for IL-6 (ELISA)

% increase above baseline

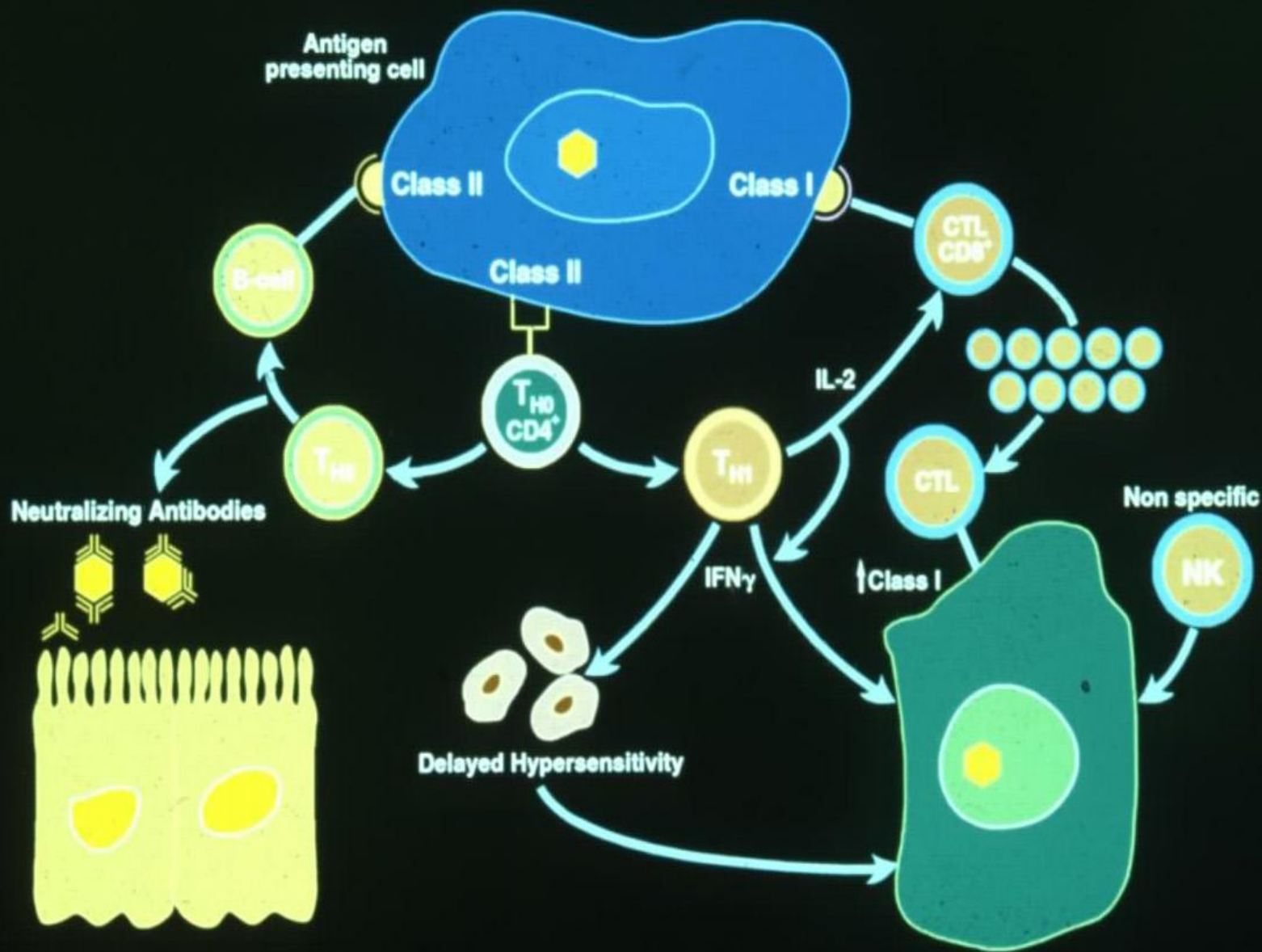




# Interleukin-6 Release from Human Airway Epithelial Cells following AdCFTR Administration



# IMMUNE RESPONSE TO ADENOVIRUS – MEDIATED GENE THERAPY

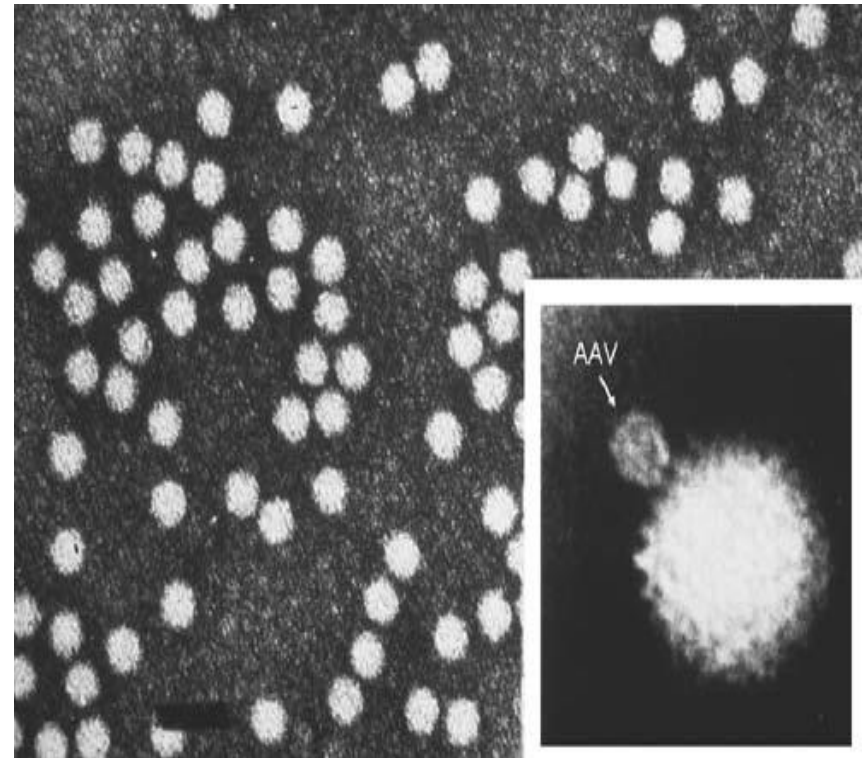


# Problems with Adenovirus

- Dose dependent inflammation cytotoxicity
- T1 and Th2 lymphocyte immunologic response
- Humoral responses making repeat administration less effective
- Gutting and stealth Ad vectors
- Inefficient in lung
  - Cellular receptor for Ad infection baso-lateral
- Patient death in 2000, OTCD

# Adeno-associated virus

- Non-enveloped icosahedron
- Linear single stranded DNA, 4.7kb
- AAV genome contains 2 genes
  - Cap encoding coat proteins
  - Rep encoding 4 regulatory proteins
  - Flanked by 2 ITR
- Transgene inserted between ITRs with deletion of viral genes
- Production of viral virions requires
  - Promoter driving transgene with polyadenylation signal flanked by ITRs
  - Plus separate plasmid containing necessary AAV and helper virus proteins



# Adeno –associated virus

- rAAV vector genomes persist longterm
- High abundance of persistent episomal forms and lack of site specific integration
- Binding and entry of rAAV2 vectors depends on interaction with attachment receptor (HSP) and co-receptor (fibroblast growth factor receptor or  $\alpha\omega\beta5$ )
- Paucity of receptors on bronchial epithelial cells

# Clinical trials rAAV

- Phase I
  - Maxillary sinuses, nasal, endobronchial, aerosol
  - Dose dependent DNA transfer, some gene expression
- Phase II
  - Multi centre, double blinded placebo controlled aerosol
  - Gene transfer detectable for c.30 days, neutralizing antibodies

# Problems with rAAV vectors

- Scarce receptors on airways
- Neutrophil elastase,
- Neutralising antibodies
- Vector persistence
  - Mainly episomal little integration
  - Wt AAV integration in chromosome 19 mediated by the rep gene in trans

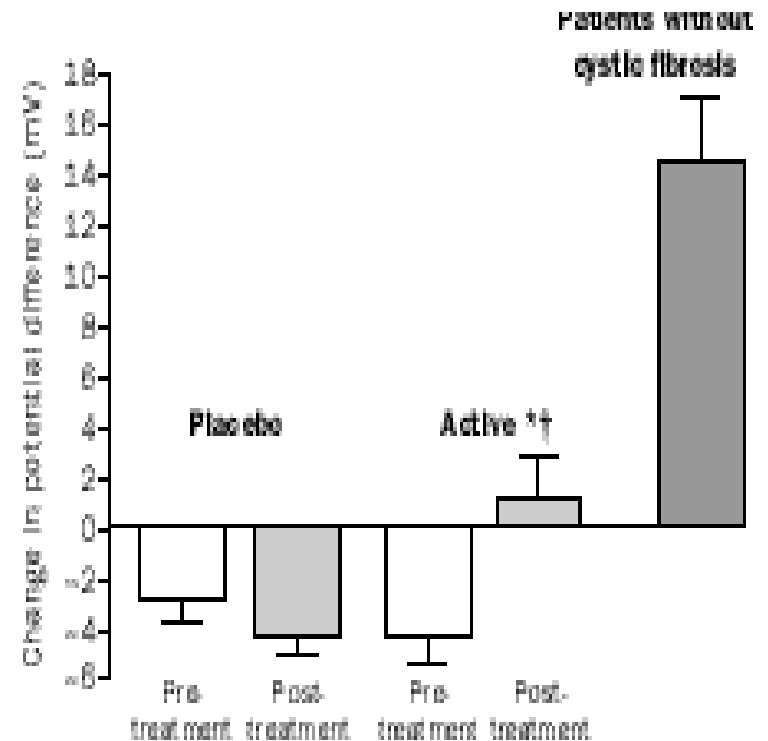
# Cationic Liposomes

- Cationic lipids usually mixed with cholesterol and dioleoyl-phosphatidylethanolamine (DOPE)
- Mixed with DNA they bind by electrostatic charge forming charged particles which interact with membranes to enter cells



# Liposome clinical trials

- 16 male CF patients
- CFTR cDNA with CMV promoter complexed to GL-67/DOPE/DMPE-PEG 5000
- Pari LC jet nebuliser
- Detected
  - Vector specific DNA
  - no vector specific CFTR mRNA
  - No significant change in baseline PD
  - No significant change in amiloride response
  - Increases in chloride conductance in treated group



# Problems with Liposomes

- Fever and increased IL6 after administration
- Attributed to innate immune response
  - TLR9 recognising bacterially derived unmethylated CpG motifs
- Further studied by Ruiz et al (2001)
  - Fever , increased IL6, 1hr post aerosol
  - PBMC responded to plasmid DNA and more so when complexed to GL-67
  - Milder response when GL-67 complexed to eukaryotic DNA

# GL67A/pGM169

cationic liposome (GL67A) and plasmid  
DNA expressing CFTR (pGM169)

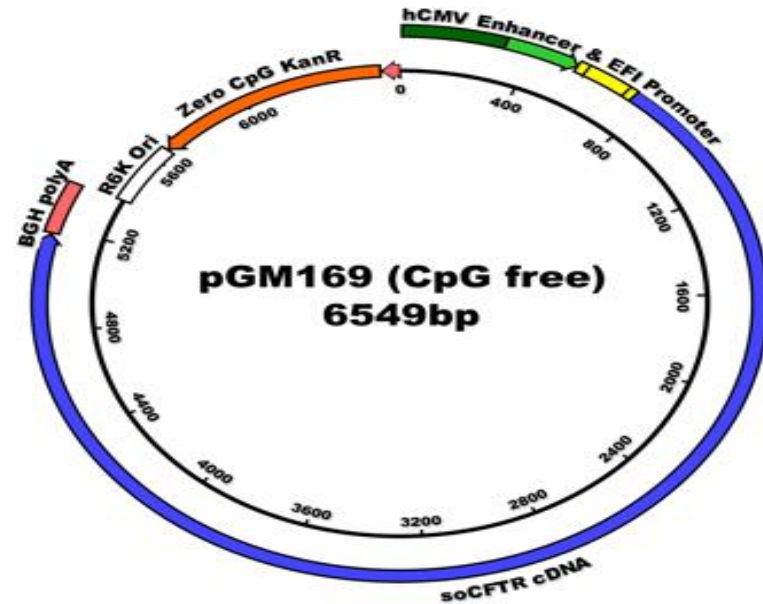
Negatively charged plasmid plus positively  
charged liposomes form tightly bound  
particles

CFTR cDNA codon optimised

hCEFI promoter

Completely devoid of CG dinucleotides

GL67 co-formulated with neutral lipid  
dioleoyl-phosphatidylethanolamine (DOPE)  
facilitates pDNA escape, along with small  
amounts of PEG-containing lipid (DMPE-  
PEG5000) stabilised at concentrations  
sufficient for aerosol delivery



# UK gene therapy consortium

- Run-in study over number of years
  - 300 PWCF
  - lung function, bacterial infection and inflammation in the lungs.
- Single dose study (2009-2011)
  - 30 patients
  - Different dosages
  - Nebuliser/some got nasal dose

# Multi dose liposome study (UK)

- 130 people with CF (aged 12 years and above)
- Blinded, placebo-controlled study
  - Medical history/clinical examination
  - Blood and urine samples
  - Spirometry (Lung function tests)
  - Sputum analysis
  - Completion of diary cards and Quality of Life Questionnaires
  - Lung clearance index (LCI)
  - Activity Monitoring via armband
  - Gas Transfer tests
  - Exercise bike tests
  - CT scans of the chest

# Problems with CFTR gene transfer

- Lungs difficult-mucus, DNA, proteases, host immune response
- Which cells: surface epithelial cells terminally differentiated-? Basal stem cells
- Efficacy difficult to assess, is nose same as bronchi?

# Other technologies

- Gene repair , splicosome mediated trans-splicing- inefficient
- Ex vivo transduction and systemic readministration of BMSCs
  - Myeloablation and extensive lung damage prerequisites for engraftment
  - Frequency of engraftment/differentiation in conducting airways very low
- Nanoparticle-mediated gene transfer

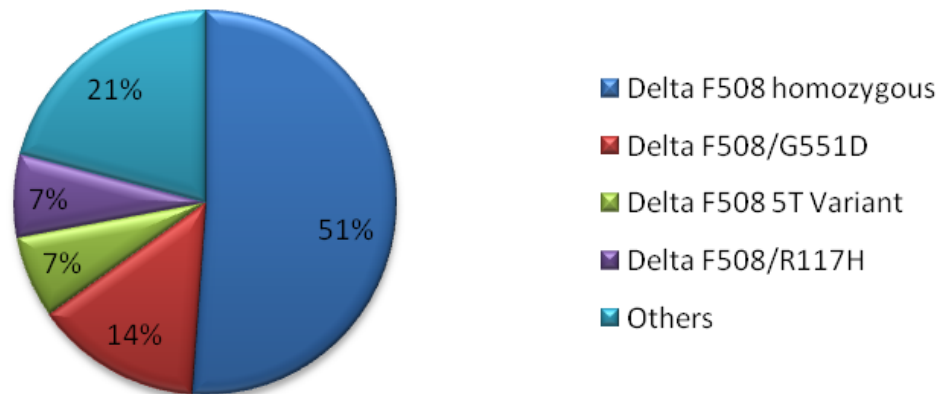
# Other viruses

- Sendai virus
- Respiratory syncytial virus (RSV)
- Human parainfluenza virus (PIV3)
  - All attach to sialic acid and cholesterol
  - Replicate in cytoplasm
- Lentivirus
  - Prolonged expression ? Integration
  - Low efficacy of gene transfer

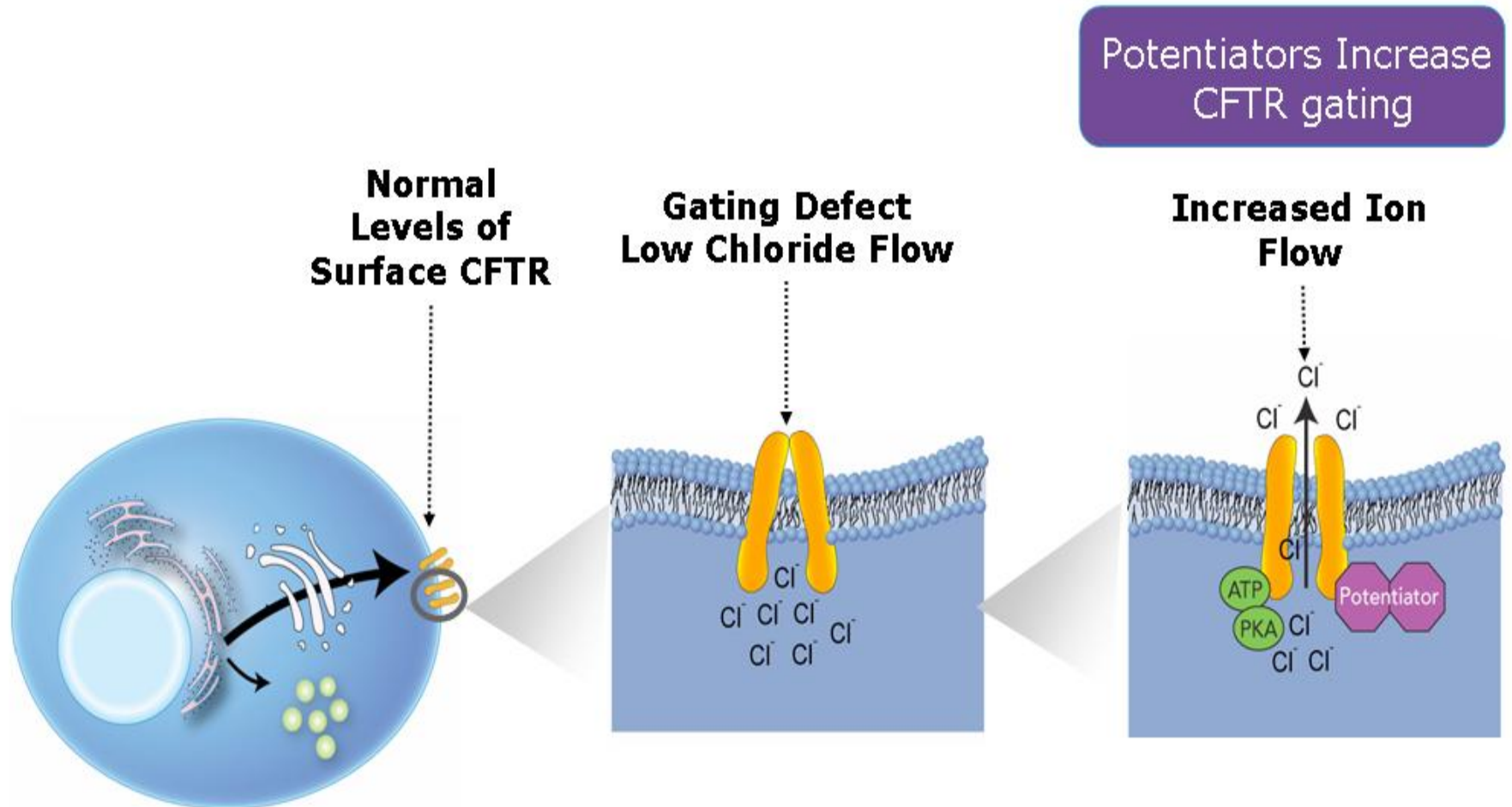


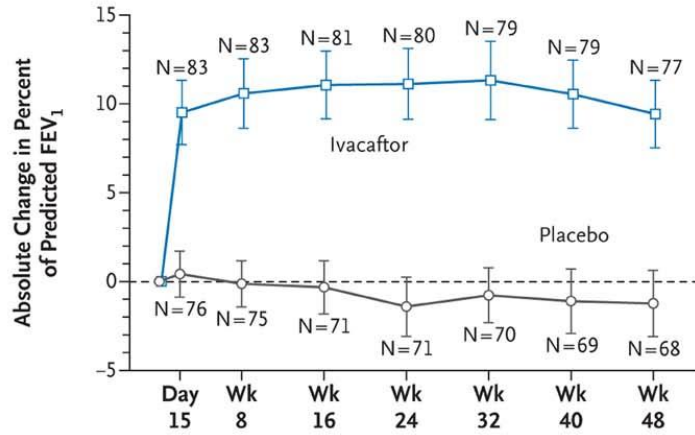
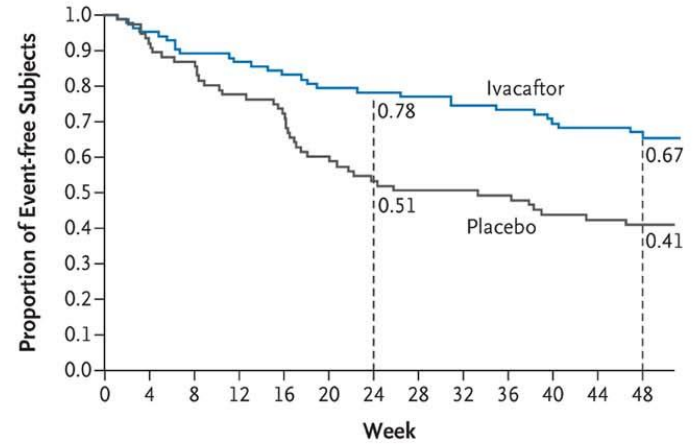
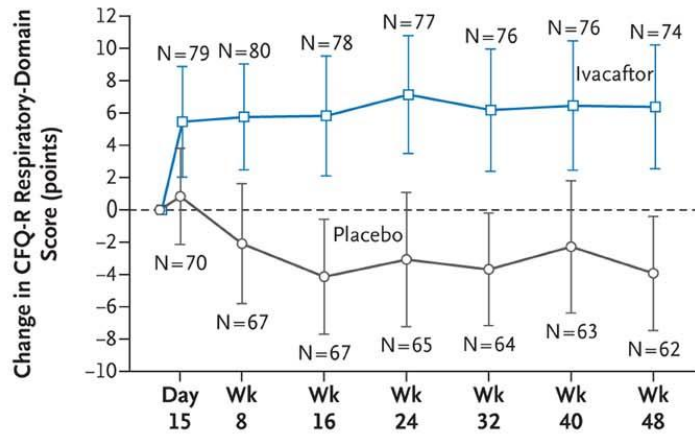
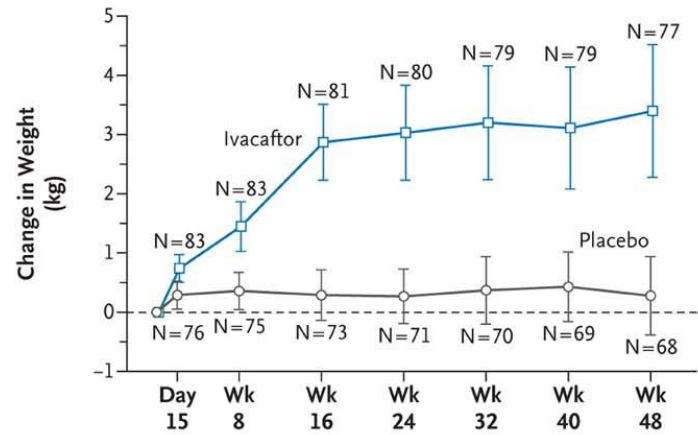
# Beaumont Hospital report 2008

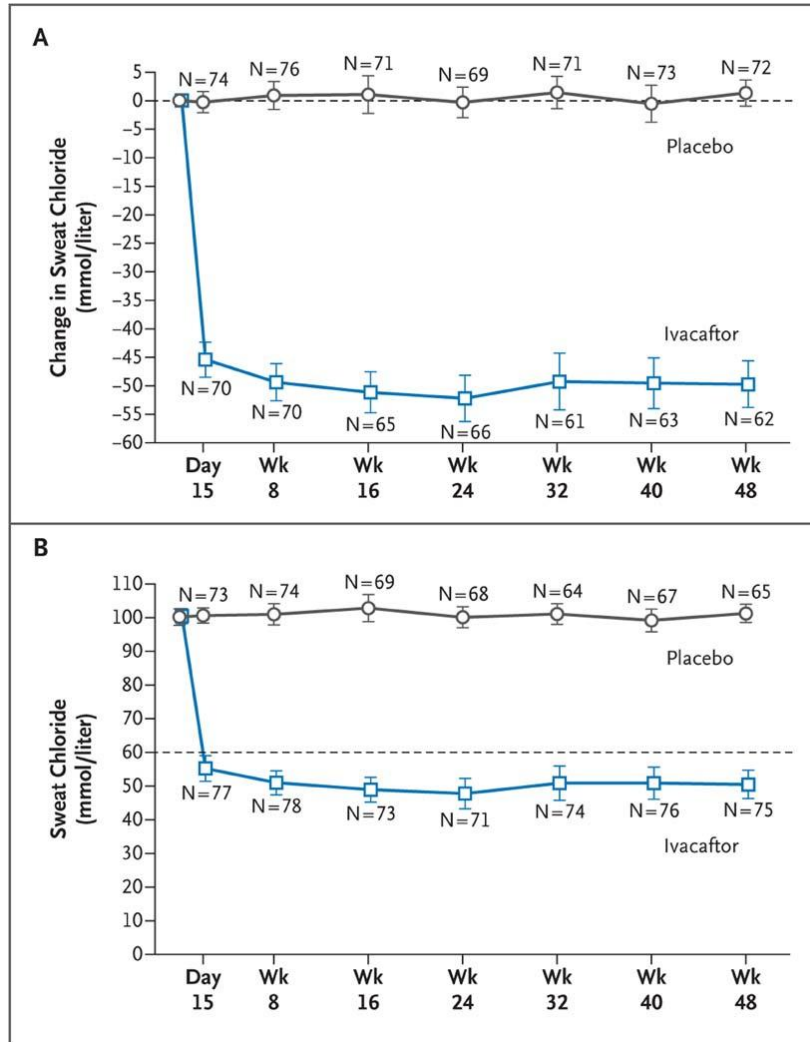
**CFTR Gene Mutations in Beaumont  
CF Patients**



# Modulators of CFTR Function



**A****B****C****D**



# CFTR function and interactions

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